

CLAIMS

We claim:

1. A nucleic acid probe for detecting hybridization of unlabeled nucleic acid comprising:  
an oligonucleotide having
  - (a) a surface-coupling group,
  - (b) a first sequence called the "loop sequence" that is complementary to a target nucleic acid sequence;
  - (c) a second and a third sequence, located on opposite ends of the loop sequence and which can hybridize to each other in the absence of the target nucleic acid sequence to form what is called a "stem structure," wherein said stem structure contains a restriction enzyme cleavage site that is not present in the loop sequence (b) when hybridized to the target nucleic acid sequence; and
  - (d) a label located on the opposite side of the restriction site from the surface-coupling group such that cleavage by restriction enzyme specific for the restriction site of the stem sequences detaches the label from the surface;wherein said loop sequence makes up all, or part, of the nucleotides between the complementary sequences, and wherein hybridization of the fully complementary target nucleic acid to the loop sequence breaks the intramolecular hybridization bonds of the stem structure and removes the restriction site.
2. The nucleic acid probe of claim 1, wherein the label is a bioluminescent, chemiluminescent, radioisotopic, isotopic, enzymatic, chromogenic, fluorescent or antibody label.

3. The nucleic acid probe of claim 2, wherein the bioluminescent label is luciferase, aequorin, alkaline phosphatase, horseradish peroxidase or  $\beta$  galactosidase.

4. The nucleic acid probe of claim 2, wherein the chemiluminescent label is acridinium.

5. The nucleic acid probe of claim 1, wherein the restriction enzyme recognition site is 4 to 8 nucleic acid bases in length.

6. The nucleic acid probe of claim 1, wherein the restriction enzyme site is specifically cleaved by a restriction enzyme selected from the group consisting of *AaaI*, *AatI*, *AatII*, *Abel*, *Abri*, *Acc16I*, *Acc65I*, *AccB1I*, *AccB2I*, *AccBSI*, *AccEBI*, *AccI*, *AccII*, *AccIII*, *AceI*, *AceII*, *AciI*, *AcpI*, *AcrII*, *AcyI*, *AeuI*, *Afa16RI*, *Afa22MI*, *AfaI*, *AfeI*, *AfII*, *AfIII*, *AgeI*, *AhaB8I*, *AhaI*, *AhaII*, *AhyI*, *AitI*, *AjoI*, *AliAJI*, *AliI*, *AluI*, *Alw21I*, *Alw44I*, *Ama87I*, *AocI*, *AocII*, *Aor13HI*, *Aor51HI*, *AorI*, *AosI*, *AosII*, *ApaCI*, *Apal*, *ApaLI*, *ApaORI*, *ApI*, *ApyI*, *AquI*, *Ascl*, *AseII*, *AsiAI*, *AsiI*, *Bce751I*, *AsiSI*, *Asp10HI*, *Asp713I*, *Asp718I*, *Asp745I*, *AspAI*, *AspHI*, *AspLEI*, *AspMDI*, *AspMI*, *AspNI*, *AspS9I*, *AstWI*, *AsuC2I*, *AsuI*, *AsuII*, *AsuIII*, *AsuNHI*, *AvaI*, *AvaII*, *Avcl*, *AvII*, *AvrBII*, *AvrII*, *AxyI*, *Bac36I*, *Bal228I*, *BalI*, *BamHI*, *BamNxI*, *BanI*, *BanII*, *BavAI*, *BavAll*, *BavBI*, *BavBII*, *BavI*, *BbeI*, *Bbi24I*, *BbiII*, *BbrPI*, *BbuI*, *Bbv12I*, *BbvAIII*, *BbvBI*, *BbvCI*, *Bca77I*, *Bce22I*, *Bce243I*, *BceBI*, *BciBII*, *BcnI*, *Bco118I*, *Bco27I*, *BcoAI*, *BcoI*, *BcuAI*, *BdiSI*, *BecAII*, *BepI*, *BetI*, *BfaI*, *Bfi57I*, *Bfi89I*, *BfmI*, *Bim19I*, *Bim19II*, *BimI*, *BliHKI*, *BlnI*, *BloHI*, *BlpI*, *BluI*, *Bme12I*, *Bme1390I*, *Bme142I*, *Bme18I*, *Bme216I*, *Bme361I*, *BmyI*, *BnaI*, *Bpu10I*, *Bpu1102I*, *Bpu14I*, *Bpu95I*, *BpuB5I*, *BpuDI*, *BpuI*, *BsaAI*, *BsaHI*, *BsaJI*, *BsaOI*, *BsaWI*, *BscBI*, *BscFI*,

Bse118I, Bse15I, Bse16I, Bse17I, Bse1I, Bse1I, Bse21I, Bse24I, Bse634I, Bse64I, BseAI, BseBI, BseDI, BseGI, BseGI, BseNI, BseNI, BsePI, BseQI, BseSI, BseT10I, BseT9I, BseX3I, Bsh1236I, Bsh1285I, Bsh45I, BshFI, BshGI, BshI, BshKI, BshNI, BshTI, BsiCI, BsiEI, BsiHKAI, BsiI, BsiKI, BsiLI, BsiMI, BsiSI, BsiWI, BsiZI, BsmSI, BsoBI, BsoCI, BsoFI, Bsp105I, Bsp119I, Bsp120I, Bsp123I, Bsp1286I, Bsp13I, Bsp143I, Bsp143II, Bsp153AI, Bsp1720I, Bsp1894I, Bsp19I, Bsp2095I, Bsp211I, Bsp4009I, Bsp50I, Bsp519I, Bsp63I, Bsp67I, Bsp68I, Bsp6I, Bsp98I, BspA2I, BspAAI, BspAAIII, BspAI, BspBI, BspBII, BspBRI, BspCI, BspEI, BspF4I, BspFI BspJI, BspKI, BspKT6I, BspLAI, BspLAI, BspLI, BspLS2I, BspM39I, BspMII, BspMKI, BspNI, BspO4I, BspR7I, BspRI, BspT104I, BspT107I, BsrAI, BsrBI, BsrFI, BsrI, BsrI, BsrSI, BsrSI, BssAI, BssECI, BssHI, BssHII, BssKI, BssSI, BssT1I, Bst100I, Bst11I, Bst11I, Bst1I, Bst2BI, Bst2I, Bst2UI, Bst31NI, Bst38I, Bst40I, Bst4CI, BstACI, BstB7SI, BstBAI, BstBI, BstBZ153I, BstD102I, BstDEI, BstDSI, BstEII, BstENII, BstF5I, BstF5I, BstFNI, BstH2I, BstHHI, BstHZ55I, BstI, BstJZ301I, BstM6I, BstMCI, BstMZ611I, BstNI, BstNSI, BstOI, BstPI, BstSFI, BstSI, BstT10I, BstT9I, BstUI, BstVI, BstX2I, BstXI, BstYI, BstZI, Bsu1532I, Bsu1854I, Bsu23I, Bsu36I, Bsu54I, BsuBI, BsuFI, BsuRI, BteI, BtgI, BthAI, BthDI, BthEI, BtkI, BtkII, BtrI, BtsI, BvuBI, BvuI, Cac8I, CacI, CauB3I, CauI, CauII, CbiI, CboI, CbrI, CciNI, CcoI, CcrI, CcuI, CcyI, CeiI, CeiII, CfiI, CfoI, Cfr10I, Cfr13I, Cfr42I, Cfr6I, Cfr9I, CfrA4I, CfrBI, CfrI, CfrJ4I, CfuII, Chal, Ctl, CpfI, CpoI, CscI, Csp45I, Csp68KI, Csp68KII, Csp68KVI, Csp6I, CspAI, CspBI, CspI, CspKVI, CstI, CthII, CviAI, CviAII, CviBI, CviJI, CviQI, CviRI, CviRII, CviTI, CvnI, DdeI, DmaI, DpnII, DraII, DsaI, DsaII, DsaIII, DsaIV, DsaV, Eae46I, EaeAI, EaeI, EagBI, EagI, EagMI, EcaI, Eci125I, EciI, Ecl136II, Ecl18kI, Ecl2zI, Ecl37kI, EclRI, EclXI, Eco130I, Eco137kI, Eco13kI, Eco147I, Eco1831I, Eco21kI, Eco24I, Eco27kI, Eco29kI, Eco47I, Eco47III, Eco52I, Eco53kI, Eco56I, Eco64I, Eco72I, Eco75KI, Eco78I, Eco81I, Eco88I,

*Eco*91I, *Eco*HI, *Eco*HK31I, *Eco*ICRI, *Eco*O109I, *Eco*O128I, *Eco*O65I, *Eco*RII, *Eco*T14I, *Eco*T38I, *Ege*I, *Ehe*I, *Erh*B9I, *Erh*B9II, *Erh*I, *Erp*I, *Esp*I, *Fau*BII, *Fb*II, *Fbr*I, *Fdi*I, *Fdi*II, *Fgo*I, *Fmu*I, *Fnu*4HI, *Fnu*AI, *Fnu*CI, *Fnu*DI, *Fnu*DII, *Fnu*DIII, *Fnu*EI, *Fri*OI, *Fse*I, *Fsp*1604I, *Fsp*4HI, *Fsp*I, *Fsp*II, *Fsp*MSI, *Fss*I, *Fun*I, *Ga*II, *Gce*GLI, *Gce*I, *Gdi*I, *Gdi*II, *Hac*I, *Hae*I, *Hae*II, *Hae*III, *Hal*II, *Hap*II, *Hgi*AI, *Hgi*BI, *Hgi*CI, *Hgi*CII, *Hgi*CIII, *Hgi*DI, *Hgi*DII, *Hgi*EI, *Hgi*GI, *Hgi*HI, *Hgi*HII, *Hgi*HIII, *Hgi*I, *Hgi*JI, *Hgi*JII, *Hgi*S22I, *Hha*I, *Hha*II, *Hin*1I, *Hin*1II, *Hin*2I, *Hin*6I, *Hinc*II, *Hind*II, *Hinf*I, *Hin*JCI, *Hin*P1I, *Hpa*II, *Hpy*178III, *Hpy*188I, *Hpy*188III, *Hpy*51I, *Hpy*8I, *Hpy*99I, *Hpy*BI, *Hpy*BII, *Hpy*CH4I, *Hpy*CH4III, *Hpy*CH4IV, *Hpy*CH4V, *Hso*I, *Hsp*92I, *Hsp*92II, *Hsp*AI, *Ita*I, *Kas*I, *Kaz*48kI, *Kox*II, *Kpn*2I, *Kpn*2kI, *Kpn*378I, *Kpn*49kII, *Kpn*I, *Ksp*I, *Kzo*49I, *Kzo*9I, *Lla*AI, *Lla*BI, *Lmu*60I, *Lpn*I, *Lsp*I, *Mab*I, *Mae*I, *Mae*II, *Mae*III, *Mae*K81I, *Mae*K81II, *Mav*I, *Mbi*I, *Mbo*I, *Mch*AI, *Mch*AII, *Mch*I, *Mcr*I, *Mf*II, *Mfo*AI, *Mgl*14481I, *Mgo*I, *Mha*AI, *Mh*II, *Mkr*AI, *Mla*AI, *Mla*I, *Mls*I, *Mlt*I, *Mlu*23I, *Mlu*31I, *Mlu*B2I, *Mlu*I, *Mlu*NI, *Mly*113I, *Mno*I, *Mro*I, *Mro*NI, *Msc*I, *Msp*17I, *Msp*67I, *Msp*A1I, *Msp*B4I, *Msp*I, *Msp*R9I, *Msp*V28I, *Msp*YI, *Mst*I, *Mst*II, *Mth*ZI, *Mva*I, *Mvn*I, *Mvr*I, *Mxa*I, *Nae*I, *Nar*I, *Nb*II, *Nci*I, *Nco*I, *Nda*I, *Nde*II, *Ngo*AIII, *Ngo*AIV, *Ngo*MIV, *Ngo*PII, *Ngo*PIII, *Nhe*I, *Nla*II, *Nla*III, *Nla*IV, *Nli*3877I, *Nme*CI, *Nme*RI, *Nmu*CI, *Nop*I, *Not*I, *Nph*I, *Nru*I, *Nsb*I, *Nsp*29132II, *Nsp*7121I, *Nsp*BII, *Nsp*HI, *Nsp*I, *Nsp*II, *Nsp*III, *Nsp*IV, *Nsp*LKI, *Nsp*SAI, *Nsp*SAII, *Nsp*SAIV, *Nsp*V, *Nun*II, *Okr*AI, *Oxa*NI, *Pac*25I, *Pae*14kI, *Pae*17kI, *Pae*5kI, *Pae*AI, *Pae*BI, *Pae*HI, *Pae*I, *Pae*PI, *Pae*QI, *Pae*R7I, *Pal*I, *Pam*I, *Pam*II, *Pan*I, *Pau*AI, *Pau*I, *Pde*12I, *Pde*133I, *Pde*137I, *Pdi*I, *Pfa*AI, *Pfa*AIII, *Pfl*21I, *Pfl*23II, *Pfl*27I, *Pfl*8I, *Pfl*KI, *Pin*AI, *Pin*BII, *Pla*AI, *Pla*II, *Pla*II, *Ple*19I, *Psu*161I, *Pma*CI, *Pme*55I, *Pmi*I, *Ppa*AI, *Ppa*AII, *Ppe*I, *Ppu*AI, *Ppu*MI, *Ppu*XI, *Psp*03I, *Psp*124BI, *Psp*23I, *Psp*5II, *Psp*AI, *Psp*ALI, *Psp*EI, *Psp*GI, *Psp*LI, *Psp*N4I, *Psp*OMI, *Psp*PI, *Psp*PPI, *Pss*I, *Psi*I, *Pst*NHI, *Psu*AI, *Psu*I, *Pta*I, *Pun*AI, *Pun*AII, *Pvu*84II, *Pvu*I, *Pvu*II, *Ral*F40I, *Rfl*FI, *Rma*I, *Rsa*I, *Rsh*I, *Rsp*LKI,

*Rsp*LKII, *Rsr*2I, *Rsr*II, *Rtr*63I, *Rtr*I, *Sac*I, *Sac*II, *Sac*NI, *Sa*II, *Sa*PI, *Sa*DI, *Sa*I, *Sa*I, *Sau*3239I, *Sau*3AI, *Sau*96I, *Sau*BMKI, *Sau*HPI, *Sau*I, *Sau*LPI, *Sau*LPII, *Sau*MI, *Sau*NI, *Sau*SI, *Sb*fl, *Sb*i68I, *Sbo*13I, *Sb*vI, *Sce*III, *Sch*ZI, *Sc*I, *Sc*NI, *Sc*FI, *Sd*I, *Sd*II, *Se*I, *Se*II, *Se*nPT14bI, *Se*nPT16I, *Sex*AI, *Sex*BI, *Sex*CI, *Sf*I, *Sf*CI, *Sf*eI, *Sf*II, *Sf*oI, *Sfr*274I, *Sfr*303I, *Sf*uI, *Sg*fl, *Sgr*AI, *Sgr*BI, *Si*MI, *Si*nI, *Sl*I, *Sle*I, *Slu*1777I, *Sma*I, *Sm*II, *Sm*uEI, *Sni*I, *Sno*I, *Sol*10179I, *Sol*II, *Spa*HI, *Sph*I, *Sp*II, *Spo*I, *Spu*I, *Sr*fl, *Sr*II, *Sru*30DI, *Ssc*L1I, *Sse*1825I, *Sse*232I, *Sse*8387I, *Sse*AI, *Sse*BI, *Ssh*AI, *Ss*II, *Sso*II, *Ssp*1I, *Ssp*5230I, *Ssp*AI, *Ssp*I, *Ssp*RFI, *Sst*I, *Sst*II, *St*eI, *St*h117I, *St*h134I, *St*h368I, *St*hI, *St*rI, *St*uI, *St*yD4I, *St*yI, *Sua*I, *Sun*I, *Sur*I, *Svi*I, *Taa*I, *Tai*I, *Taq*52I, *Taq*I, *Taq*XI, *Tau*I, *Tha*I, *Th*I, *Tru*201I, *Tsc*I, *Tse*I, *Tsp*1I, *Tsp*1I, *Tsp*32I, *Tsp*32II, *Tsp*45I, *Tsp*49I, *Tsp*4CI, *Tsp*BI, *Tth*HB8I, *Unb*I, *Uur*960I, *Vne*I, *Vpa*K11AI, *Vpa*K11BI, *Xce*I, *Xci*I, *Xcy*I, *Xho*I, *Xho*II, *Xma*CI, *Xma*I, *Xma*III, *Xma*JI, *Xmi*I, *Xor*II, *Xpa*I, *Xsp*I, *Yen*I, and *Zan*I.

7. The nucleic acid probe of claim 1, wherein the surface coupling group is a covalent coupling group.

8. The nucleic acid probe of claim 1, wherein the label is attached to the oligonucleotide through a biotin-streptavidin coupling.

9. The nucleic acid probe of claim 1, wherein the oligonucleotide contains spacer groups at either or both ends.

10. The nucleic acid probe of claim 9, wherein the spacer groups are polythymine spacers.

11. The nucleic acid probe of claim 7, wherein the covalent surface coupling group is an aminopropanol moiety.

12. The nucleic acid probe of claim 7, wherein the oligonucleotide is attached to a surface with the covalent coupling group.

13. The nucleic acid probe of claim 12, wherein the surface is a glass, silicon, gold, metal or plastic surface.

14. The nucleotide probe of claim 1, wherein the loop sequence is 16 to 25 nucleotides in length.

15. A method of determining the presence of a target nucleic acid comprising:  
exposing a sample to a nucleic acid probe of claim 1 under effective conditions for specific hybridization of the target nucleic acid to the nucleic acid probe;  
adding restriction enzyme specific for the restriction site contained in the stem structure and under conditions to cleave the nucleic acid probe with the restriction enzyme;  
determining the presence of the target nucleic acid, wherein the presence or amount of label is indicative of the presence of target nucleic acid.